CHAPTER 2

Adverse renal effects of ACE inhibition during dietary sodium restriction in proteinuric and healthy rats

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Abstract

Angiotensin-converting enzyme inhibitors (ACEi) provide renoprotection. A low sodium diet enhances their efficacy. However, the added effect of sodium restriction on proteinuria and blood pressure is not invariably associated with better preservation of renal morphology, suggesting that the combination of ACEi with low sodium can elicit renal structural abnormalities. To test this hypothesis we investigated the effects of ACEi in combination with a control (CS) or a low sodium (LS) diet in healthy and nephrotic rats. After three weeks of treatment rats were sacrificed and kidneys examined for renal structural abnormalities.

In healthy rats ACEi reduced blood pressure: the fall in blood pressure was significantly larger in the ACEi/LS group. In the ACEi/CS group renal morphology was normal, but in the ACEi/LS group severe interstitial damage was found. This was associated with increased interstitial macrophage influx, and upregulation of osteopontin, alpha-SMA and collagen III expression. In addition ACEi/LS induced an increase in total medial area of afferent arterioles. In nephrotic rats ACEi/LS reduced both blood pressure and proteinuria, whereas in the ACEi/CS group only blood pressure was reduced. In the ACEi/CS group mild interstitial damage was present, but strikingly, in the ACEi/LS group pronounced tubulo-interstitial abnormalities occurred, similar to those seen in ACEi/LS healthy rats, with similar changes in afferent arteriolar wall.

In conclusion, the combination of ACEi/LS elicits pronounced renal interstitial abnormalities in healthy and nephrotic rats, despite significant reduction of proteinuria in the latter. Considering their occurrence in healthy rats, these renal adverse effects cannot be due to specific particularities of adriamycin nephrosis. Further studies should elucidate the mechanisms underlying these observations and their impact on long term renoprotection.

Introduction

Intervention in the renin-angiotensin aldosterone system (RAAS) by Angiotensin-converting enzyme inhibitors (ACEi) provides renoprotection in experimental and human chronic renal disease by its beneficial effects on blood pressure, glomerular hemodynamics, and particularly proteinuria[1,2]. The response to ACEi is modified by sodium intake, with a blunted response during high sodium, and an enhanced response during low sodium intake. The added effects of low sodium intake apply to blood pressure as well as proteinuria[3-5] in human and experimental conditions[6-8]. Sodium restriction is therefore recommended during ACEi to enhance the renoprotective benefit.

However, the added effects of sodium restriction on the intermediate parameters proteinuria and blood pressure are not invariably associated with better preservation of renal morphology in experimental adriamycin nephrosis[9]. Dissociation between the anti-proteinuric effect of ACEi and lack of protection against structural abnormalities has also been observed in experimental transplantation, with failure to affect interstitial lesions. In the latter study ACEi was also associated with induction of preglomerular vascular lesions[10]. Together, these data suggest that ACEi, be it or not in combination with low sodium, can induce renal structural abnormalities, despite reduction of proteinuria. It is unknown whether these renal abnormalities are attributed to specific characteristics of the experimental model or to the ACEi regimen itself. Therefore, we investigated whether ACEi in combination with a normal or a low sodium intake can induce renal structural abnormalities in nephrotic and in healthy rats.

Material and Methods

Animals and Study design

Male Wistar rats (250-300g, Harlan, Zeist, The Netherlands) were housed under standard conditions at the animal research facility with free access to drinking solution and rat chow. Experiments were in accord with institutional and legislator regulations and approved by the local Committee for Animal Experiments. After one week adaptation, rats were allocated to one of the two studies (Figure 1).

Healthy rats. In the first study 40 healthy rats were randomly divided to receive either the ACEi lisinopril (Merck, Sharp & Dohme, Rahway, NJ, USA) or vehicle for three weeks, combined with either a control diet (CS, 2.0% NaCl, Hope Farms, Woerden, The Netherlands) (n=10) or a low sodium diet (LS, 0.05% NaCl, Hope Farms) (n=10).

Proteinuric rats. In the second study, we used our normotensive model of established adriamycin nephrosis (AN)[6,11], a well-characterized model of progressive renal damage induced by one tail vein injection of adriamycin (1.5 mg/kg). Proteinuria develops gradually, resulting in renal damage with a relatively large variability between individual animals[6,11,12] comparable to human renal disease. In our routine set-up interventions start six weeks after disease induction, at established proteinuria. At that time rats were stratified according to proteinuria and divided into

two groups (n=12-14 per group). Both groups received lisinopril for three weeks combined with a CS diet or a LS diet with similar sodium concentrations as described for healthy rats. We established previously that a CS or LS diet as such does not modulate renal damage in AN[13]. However, for reference a separate AN control group was included from a different experiment. This group received a LS diet only. Because of batch differences this group was not included as a full study group, but only served to provide values for morphological changes induced by adriamycin nephrosis. Lisinopril was provided at a dose of 75 mg/L in drinking water, causing maximal reduction of proteinuria in AN[6].

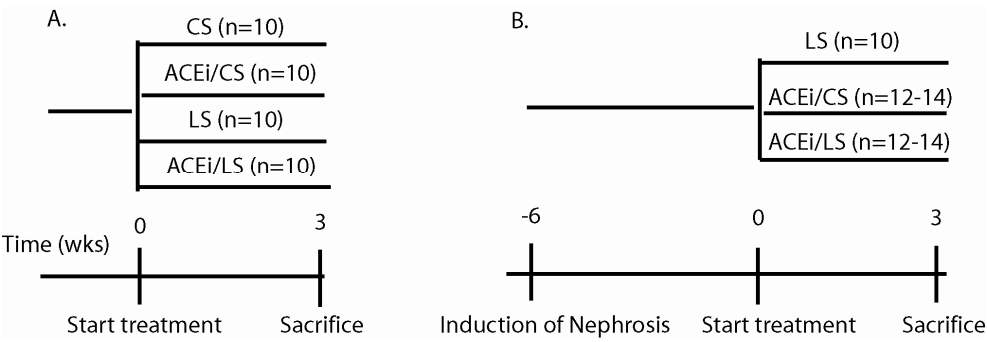

Figure 1. Study design A. *Healthy rats received vehicle or the ACE inhibitor lisinopril (ACEi) for three weeks combined with either a control sodium diet (CS, 2.0% NaCl) or a low sodium diet (LS, 0.05% NaCl).* ***B.*** *Adriamycin nephrosis (AN) was induced and 6 weeks later at established proteinuria, treatment was started. Rats received an ACEi for three weeks. ACEi was combined with either a control sodium diet (CS, 2.0% NaCl) or a low sodium diet (LS, 0.05% NaCl). For reference, a separate AN control group (LS, 0.05% NaCl, n=10) was included from a different experiment.*

Measurements

Body weight was measured weekly and rats were placed in metabolic cages for collection of 24h-urine samples and analyses of urinary sodium content. Urinary concentration of protein was determined with the biuret method (Bioquant™; Merck, Darmstadt, Germany). Creatinine was determined by a multi-analyzer (SMA-C®; Technicon, Tarrytown, NY, USA). After two weeks of daily training, systolic blood pressure was measured in conscious animals using the tail-cuff method (Apollo 179; IITC Life Science, Woodland Hills, California). The mean of three consecutive measurements was taken as the final value.

At sacrifice, rats were anesthetized with 1.5% isoflurane in N_2O/O_2. The aorta was cannulated and blood samples were taken. Kidneys were harvested after perfusion with saline. Coronal kidney slices were fixed with 4% paraformaldehyde for paraffin embedding or snap frozen in liquid nitrogen and stored at -80°C.

Immunohistochemical staining procedures

Deparaffinized sections (4µm) were stained with periodic acid-Schiff (PAS) to evaluate renal morphology. For immunostaining, sections were subjected to heat-induced antigen retrieval by overnight incubation in 0.1M Tris/HCL buffer (pH 9) at 80°C. For renin staining sections were subjected to protease (0.1%) treatment for 30 minutes at room temperature. Endogenous peroxidase was blocked with 0.075% H_2O_2 in phosphate buffered saline (PBS, pH 7.4) for 30 minutes. Macrophages (ED1), osteopontin (OPN), a marker for early tubular damage, and the pre-fibrotic marker for myofibroblast transformation alpha-smooth muscle actin (α-SMA) were detected using murine monoclonal antibodies (ED1; Serotec Ltd, Oxford, UK) (OPN; clone MPIIIB10, Developmental Hybridoma Studies Institute, Iowa City, USA) (α-SMA; clone 1A4, Sigma, St. Louis, MO, USA) for 60 min at room temperature. Binding was detected using sequential incubations with peroxidase-labeled rabbit anti-mouse and peroxidase-labeled goat anti-rabbit antibodies (Dakopatts, Glostrup, Denmark) for 30 min.

Collagen III and renin were detected using polyclonal antibodies (Col III; Biogenesis, Ltd, Poole, UK) (Renin antibody, a gift from Dr. T. Inagami, Vanderbilt University School of Medicine, Nashville, USA) for 60 minutes at room temperature. Binding was detected using sequential incubations with peroxidase-labeled goat anti-rabbit and peroxidase-labeled rabbit anti-goat (Dakopatts, Glostrup, Denmark) for 30 minutes.

All antibody dilutions were made in PBS supplemented with 1% BSA, 1% normal rat serum was added to the secondary antibodies. Peroxidase activity was developed by using 3,3'-diaminobenzidine tetrachloride for 10 min containing 0.03% H_2O_2. Counterstaining was performed using Mayer's hematoxylin.

Glomerular and interstitial changes

Sections were examined in a blinded fashion. The incidence and severity of focal and segmental glomerulosclerosis (FGS) were assessed by semi-quantitative scoring of 50 glomeruli per slide semi-quantitatively on a scale of 0 to 4. FGS was scored positive when mesangial matrix expansion and adhesion of the glomerular visceral epithelium to Bowman's capsule were simultaneously present. If 25% of the glomerulus was affected, a score of 1 was adjudged, 50% was scored as 2 etc. The ultimate severity score is obtained by multiplying the degree of change by the percentage of glomeruli with the same degree of injury and adding these scores. Glomerular volumes were measured as described previously[14]. Glomerular and interstitial macrophages were manually counted in respectively 50 glomeruli and 30 interstitial fields. The mean number per field was then calculated. The extent of interstitial α-SMA, osteopontin and collagen III protein expression was measured by using computer-assisted morphometry. Forty cortical interstitial fields were measured, excluding arteries and glomeruli. The surface area

found positive was divided by the total area of the field, providing a percentage of positive tissue. The average score of all interstitial fields per section was calculated.

RNA isolation and realtime RT-PCR for HO-I

RNA extraction, DNAse treatment, cDNA synthesis and qPCR analysis based on the TaqMan methodology were performed as described previously [15]. HO-1 gene-specific Taqman probe and primer sets were obtained from Applied Biosystems as Assays-on-Demand (AOD) gene expression products HO-1 Rn 00561387 m1.

Afferent arterioles

To quantify interlobular artery and afferent arteriolar wall thickness computerized morphometrical measurements were performed on renin stained sections counterstained with PAS. Interlobular arteries were identified as a single muscular artery within the inner cortex and, at times, lying close to the glomerulus and were easily distinguished from afferent arterioles. Afferent arterioles were situated in the cortex and fulfilled at least one of the following criteria: 1) presence of an internal elastic lamina, which is absent in efferent arterioles; 2) a close relation to a glomerulus; 3) presence of renin positive cells. For each arteriole and artery, the outline of the vessel and its internal lumen (excluding the endothelium) was generated using computer analysis to calculate the total medial area (outline-inline). Branching vessels and arteries that were not sectioned transversely (i.e. wall thickness was asymmetrical and a measured roundness > 1.25) were excluded. From each animal 15 to 20 afferent arterioles were evaluated.

Statistics

All data are expressed as mean ± standard deviation. Differences between rats treated with or without ACEi were analysed with a non-parametric Kruskal-Wallis test followed by a Mann–Whitney U test with correction for multiple comparisons. In proteinuric rats Wilcoxon's non-parametric test for paired samples was used to compare parameters from baseline with sacrifice. Statistical significance was accepted at $P<0.05$.

Results

Rat characteristics

Healthy rats. Clinical parameters at sacrifice are shown in table 1. A considerable difference in sodium status was obtained by the different diets as reflected by urinary sodium excretion, which was significantly higher in CS rats than in LS fed rats. This was most pronounced in LS rats treated with lisinopril (ACEi/LS). ACEi was associated with higher sodium excretion in CS fed rats. LS per se did not affect body weight or SBP, which is in accordance with a normal functioning of regulatory mechanisms of the renal-body fluid system for arterial pressure control. ACEi reduced

SBP significantly in CS and LS groups; although more pronounced in the latter. Body weight was significantly reduced by ACEi/LS.

Proteinuric rats. Table 2 shows the clinical parameters at baseline (6 weeks after disease induction) and after three weeks of intervention with ACEi. Body weight was reduced by ACEi/LS. SBP was significantly decreased by ACEi in CS and LS rats. Proteinuria, however, was only reduced in ACEi/LS rats. Plasma creatinine was not affected by ACEi.

Table 1. Characteristics of healthy rats after three weeks of ACE inhibition

Healthy Rats		Urinary sodium (mg/24h)	Body weight (g)	Systolic blood pressure (mm Hg)
CS	3 wks	3.37 ± 0.3	398 ± 10	140 ± 4
ACEi/CS	3 wks	4.91 ± 0.4*	398 ± 11	125 ± 4*
LS	3 wks	0.60 ± 0.1#	398 ± 12	143 ± 6
ACEi/LS	3 wks	0.55 ± 0.1#	312 ± 12*#	102 ± 2*#

*Rats received a control sodium (CS, 2.0% NaCl) or a low sodium (LS, 0.05% NaCl) diet, which was combined with the ACE inhibitor (ACEi) lisinopril (75 mg/l drinking water). Data are expressed as mean ± SD (n= 10 per group).*P<0.01 ACEi/LS vs. LS and ACEi/CS vs. CS. #P<0.001 LS vs. CS and ACEi/LS vs. ACEi/CS.*

Table 2. Characteristics of adriamycin nephrotic rats after three weeks of ACE inhibition

Proteinuric rats		Urinary sodium (mg/24h)	Body weight (g)	Systolic blood pressure (mm Hg)	Urinary protein (mg/24h)	Plasma creatinine (mg/dL)
ACEi/CS	Baseline	0.81 ± 0.37	419 ± 21	149 ± 17	317 ± 218	0.45 ± 0.09
	3 wks	4.08 ± 1.70*	454 ± 23*	111 ± 19*	351 ± 239	0.46 ± 0.13
ACEi/LS	Baseline	0.65 ± 0.24	412 ± 20	152 ± 12	288 ± 207	0.43 ± 0.08
	3 wks	0.44 ±0.16*#	393 ± 30*#	103 ± 18*	48 ± 17*#	0.49 ± 0.14

*Characteristics of adriamycin nephrotic rats at established proteinuria, i.e. 6 weeks after induction of nephrosis (baseline) and after three weeks of ACEi. Rats received a control sodium diet (CS, 2.0% NaCl) or a low sodium diet (LS, 0.05% NaCl) which was combined with the ACE inhibitor (ACEi) lisinopril (75 mg/l drinking water). Data are expressed as mean ± SD (n=12 per group). *P<0.001 three weeks vs. baseline. #P<0.001 ACEi/LS vs. ACEi/CS.*

Renal structural abnormalities

Healthy rats. Glomerular structural changes for healthy rats are shown in table 3. FGS was absent in all groups. Glomerular macrophage influx and glomerular size were significantly decreased in ACEi/LS treated rats. The renal interstitium was normal in vehicle treated rats on either diet, and in ACEi/CS rats. However, in ACEi/LS group severe interstitial lesions were observed in the inner cortex and outer medulla (Figure 2A-D), characterized by tubular dilatation and atrophy, loss of tubular basement membrane integrity and interstitial fibrosis, coinciding with increased influx of interstitial macrophages (Figure 4A), which were found in high numbers

surrounding damaged tubules. Increased interstitial alpha-SMA was found in ACEi/LS rats (Figure 4B), indicating interstitial myofibroblast transformation. Abundant *de novo* osteopontin expression was found in dilated and damaged cortical proximal and distal tubules of ACEi/LS rats (Figure 4C). Some tubules with a normal morphologic appearance were positive for OPN as well (Figure 3). Consistent with the interstitial fibrosis found in the PAS staining, we noted significant interstitial accumulation of collagen III in ACEi/LS rats only (Figure 4D). Collagen III was predominantly localized in interstitial areas with damaged tubules (Figure 6).

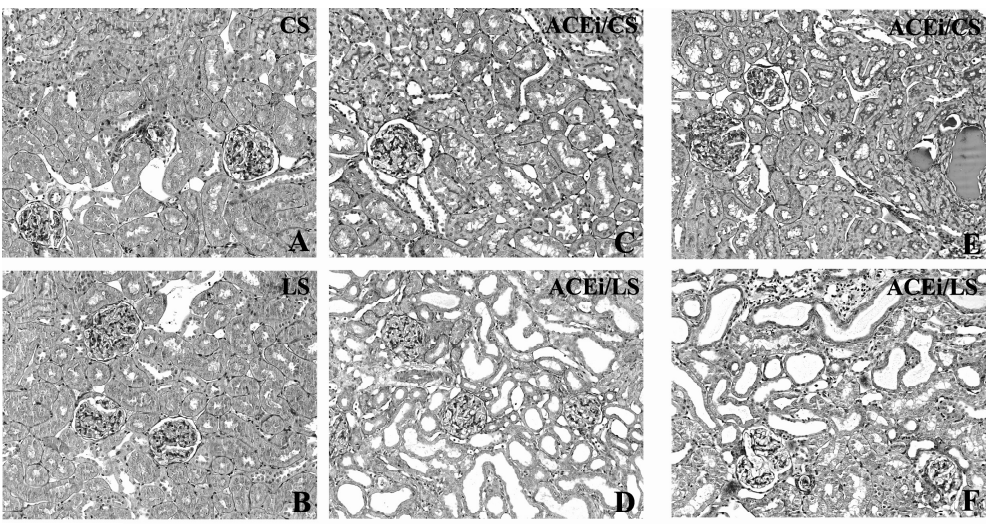

Figure 2. *Periodic acid-Schiff (PAS) staining of renal interstitium of healthy rats* ***(A-D)*** *and proteinuric rats* ***(E-F)****. Severe interstitial lesions were observed in kidneys from ACEi/LS rats. These lesions were characterized by tubular dilatation and atrophy, loss of tubular basement membrane integrity and interstitial fibrosis.*

Proteinuric rats. In AN rats mild glomerular changes were present in all groups (shown in table 3). FGS tended to be lowest in ACEi/LS rats. ACEi/LS reduced glomerular macrophage influx significantly. Glomerular size tended to be smaller in ACEi/LS treated rats compared to LS and to ACEi/CS. Predominantly in LS and ACEi/CS rats protein casts were seen in some tubules. As expected, interstitial damage was present in the untreated LS group and only barely in ACEi/CS rats. However, in ACEi/LS rats severe interstitial lesions were present in the inner cortex and outer medulla, strongly resembling those in the above-described healthy rats on the same regimen (Figure 2E-F). This coincided with an increased influx of interstitial macrophages (Figure 5A). In ACEi/LS rats interstitial alpha-SMA was decreased in comparison to the untreated LS rats, but significantly increased compared to ACEi/CS rats (Figure 5B). Osteopontin was similar in untreated LS rats and ACEi/LS rats (Figure 5C). Interstitial collagen III in ACEi/LS was increased when compared to ACEi/CS rats, and similar to untreated LS rats (Figure 5D).

Table 3. Glomerular structural changes

	FGS (score)	Incidence of FGS (%)	Glomerular macrophage influx (n)	Glomerular size ($\mu m^3 x 10^3$)
Healthy rats				
CS	0	0	1.6 ± 0.6	429 ± 57
ACEi/CS	0	0	2.4 ± 1.0	480 ± 74
LS	0	0	1.3 ± 0.3	399 ± 83
ACEi/LS	0	0	0.8 ± 0.4*#	317 ± 58#
Proteinuric rats				
LS	15.9 ± 27.0	10.8 ± 16.2	1.6 ± 0.4	517 ± 57
ACEi/CS	6.3 ± 7.1	4.0 ± 3.9	1.9 ± 0.9	565 ± 172
ACEi/LS	2.8 ± 3.6	2.0 ± 1.9	0.7 ± 0.3*#	444 ± 83

*Glomerular structural changes of healthy and proteinuric rats after three weeks of treatment. The rats received a control sodium (CS, 2.0% NaCl) or a low sodium (LS, 0.05% NaCl) diet which was combined with either vehicle or the ACE inhibitor (ACEi) lisinopril. Data are expressed as mean ± SD (n=10 per group for healthy rats and n=12 per group for proteinuric rats). *P<0.05 vs. LS. #P<0.01 vs. ACEi/CS.*

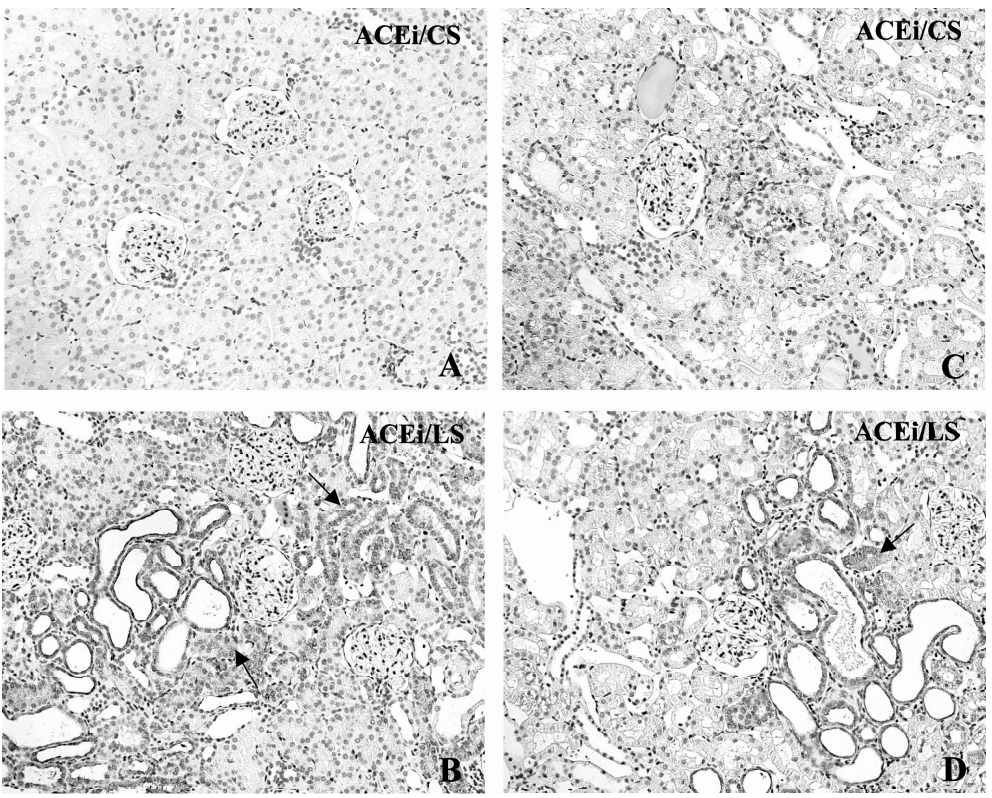

Figure 3. *Interstitial osteopontin (OPN) expression in healthy rats **(A and B)** and in proteinuric rats **(C and D)**. Abundant de novo expression of OPN was found in dilated tubules of ACEi/LS rats **(B and D)**. Some tubuli with a normal morphological appearance also showed positive OPN staining.*

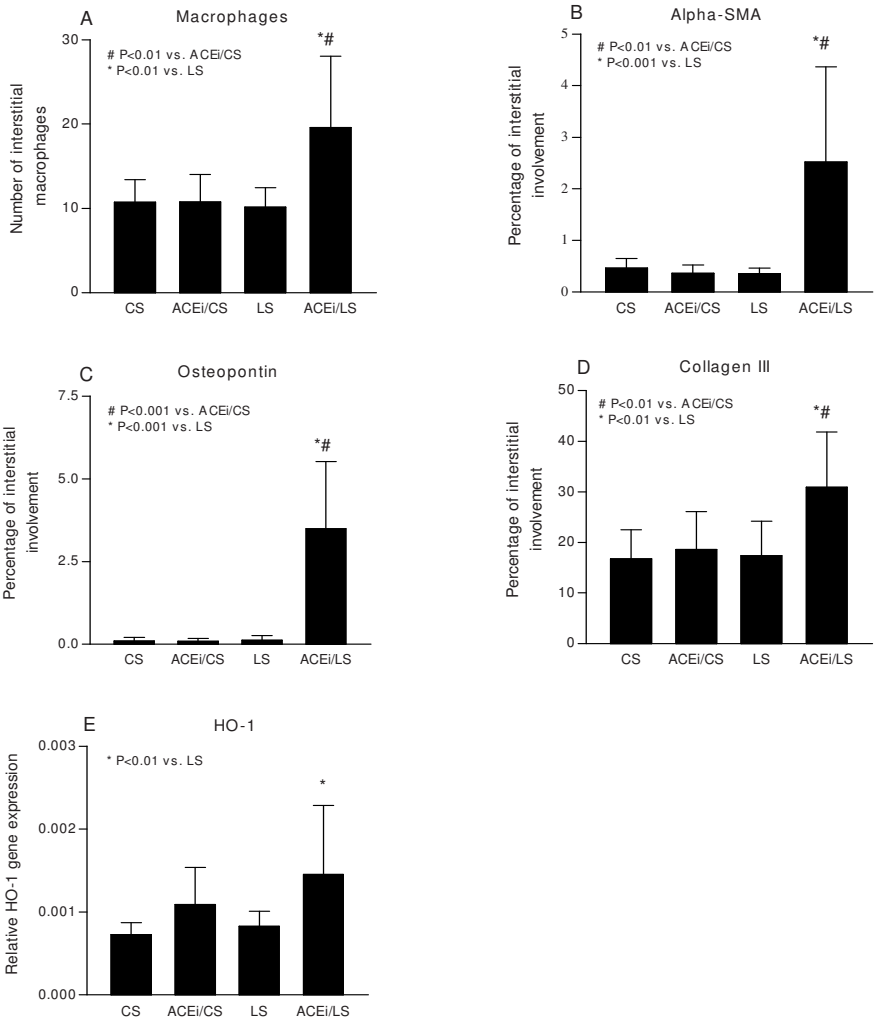

Figure 4. Renal structural markers in healthy rats. *All data are expressed as mean ± SD.* ***A.*** *Interstitial inflammation (macrophage influx) was significantly increased in ACEi/LS compared to LS and ACEi/CS.* ***B.*** *Interstitial alpha-SMA expression was upregulated by ACEi/LS.* ***C.*** *Interstitial osteopontin expression in healthy rats was increased by ACEi/LS.* ***D.*** *Interstitial fibrosis measured by collagen III expression was highest in ACEi/LS treated rats.* ***E.*** *HO-1 mRNA expression was significantly increased in ACEi/LS rats when compared to LS rats.*

HO-1 mRNA

Healthy rats. The hypoxia marker HO-1 mRNA was significantly increased in ACEi/LS rats when compared to LS rats (Figure 4E).

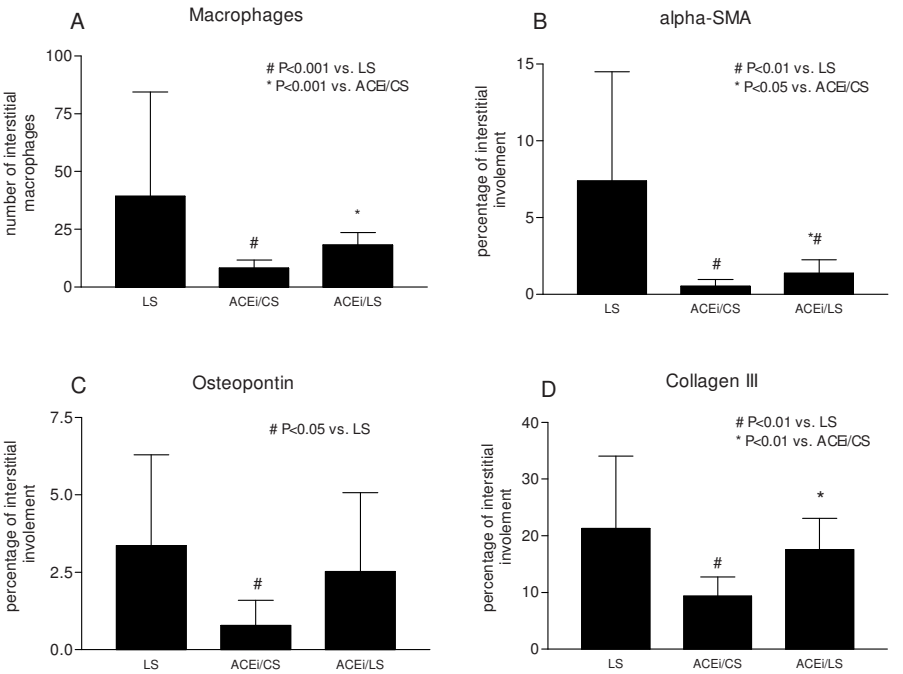

Figure 5. Renal structural markers in adriamycin nephrotic rats. *All data are expressed as mean ± SD. The LS group is a separate study group.* ***A****. Interstitial inflammation (macrophage influx) was significantly increased in ACEi/LS compared to ACEi/CS.* ***B.*** *Interstitial alpha-SMA expression was upregulated by ACEi/LS.* ***C.*** *Interstitial osteopontin expression was similar in LS and ACEI/LS rats.* ***D.*** *Interstitial fibrosis measured by collagen III expression was significantly higher in ACEi/LS than in ACEi/CS rats, but comparable to LS rats.*

Afferent arterioles

Healthy rats. Healthy rats treated with vehicle on either diet have normal renal afferent arterioles with approximately one or two renin producing granular cells in the distal part of the arteriole, the juxtaglomerular apparatus (Figure 7). ACEi/CS increased total medial area of afferent arterioles, which coincided with an increased number of cells expressing renin in the afferent arteriolar wall and a redistribution of renin upstream from the glomerulus in afferent arterioles. This effect is significantly more pronounced in ACEi/LS rats (Figure 7). The entire wall of the distal part of the afferent arteriole now consists of renin positive cells. This effect of ACEi on total medial wall area was absent in interlobular arteries (Figure 7C).

Proteinuric rats. ACEi/CS significantly increased total medial area of afferent arterioles when compared to untreated LS rats (Figure 8). This effect is significantly more pronounced in ACEi/LS rats (Figure 8). No effect of ACEi was seen on interlobular arteries (Figure 8C).

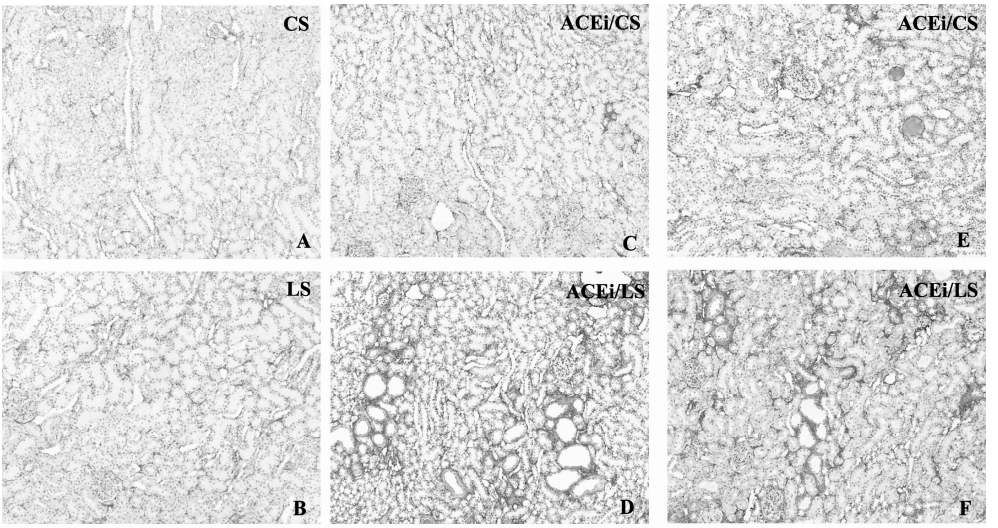

Figure 6. *Interstitial collagen III expression in healthy rats* ***(A-D)*** *and proteinuric rats* ***(E-F).*** *In animals treated with ACEi/LS accumulation of collagen III was predominantly found in interstitial areas with damaged tubules.*

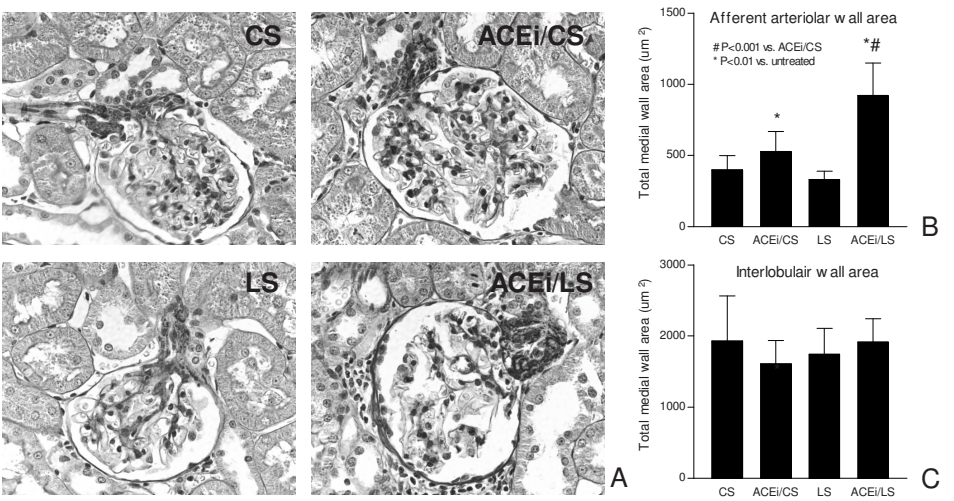

Figure 7A. Afferent arterioles of healthy rats. B. Afferent arteriolar medial wall thickness. *ACEi increased total medial wall area of the afferent arterioles of CS treated rats* ***(B).*** *This coincided with an increased number of cells expressing renin* ***(A).*** *This effect was significantly pronounced in ACEi/LS animals* ***(A and B). C. Interlobular medial wall thickness.*** *No effect of ACEi or sodium intake was seen on interlobular arterial wall thickness. All data are expressed as mean ± SD.*

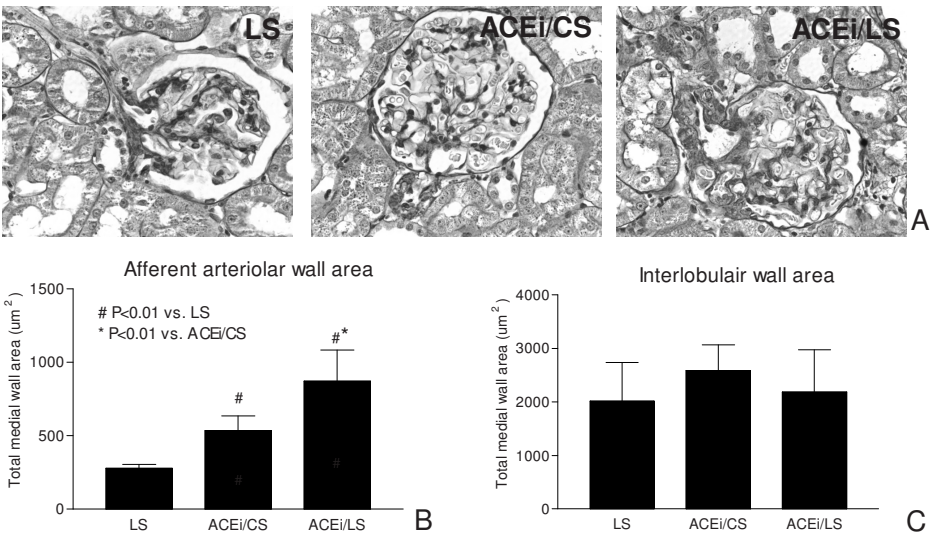

Figure 8A. ***Afferent arterioles of proteinuric rats. B. Afferent arteriolar medial wall thickness****. Total medial wall area was significantly increased by ACEi/LS.* ***C. Interlobular medial wall thickness.*** *No effect of ACEi was seen on interlobular arterial wall thickeniss. All data are expressed as mean ± SD.*

Discussion

This study shows that the combination of ACEi/LS in proteinuric rats elicits pronounced interstitial abnormalities, despite a significant reduction of proteinuria. As similar effects were found in healthy rats, the interstitial abnormalities cannot be attributed to specific characteristics of adriamycin nephrosis, but appear to be related to the regimen as such.

The renoprotective effects of ACEi in proteinuric kidney disease are well-established[1,2,16]. As reduction of proteinuria is a main factor in their renoprotective properties, the anti-proteinuric efficacy is a main surrogate parameter for renoprotection. Volume depletion by a low sodium diet or a diuretic enhances the effects of ACEi on blood pressure and proteinuria[9,17]. This combination is therefore assumed to provide better long term renoprotection. Our data in nephrotic rats confirm the effect of sodium status on the anti-proteinuric and glomeruloprotective effects of ACEi. However, the reduction of proteinuria and blood pressure was not associated with a reduction of renal interstitial damage, but with a striking increase in tubulo-interstitial (pre-)fibrotic changes. Interstitial damage in adriamycin nephrosis is attributed to toxic effects of proteinuria on the tubulo-interstitium[18], and reduction in proteinuria should thus protect against tubulointerstitial damage. Our data suggest that the combination of ACEi/LS itself exerts effects that overruled the beneficial effects of proteinuria reduction. Our study design cannot dissect whether the intrarenal changes are due to the combination of low blood pressure and the ACEi, enhanced ACEi due to low sodium or due to the lower blood pressure perse. If the lower blood pressure in combination with ACEi would be involved, our data might

provide a possible, albeit hypothetical explanation why the REIN II study did not find added benefit of a lower blood pressure in proteinuric patients on ACEi[19].

To exclude the possibility that the interstitial effect of ACEi/LS in nephrotic rats was related to specific tubulointerstitial vulnerability inherent to this model we also studied healthy rats. Remarkably, healthy rats treated with ACEi/LS developed interstitial lesions resembling those of nephrotic rats. As the interstitium was not affected by ACEi on the CS regimen or by LS itself, the combination of ACEi with LS appears to be the culprit and not the disease.

It is remarkable that the interstitial changes occurred in spite of clear-cut protective effects on the surrogate parameters blood pressure and proteinuria. Renal abnormalities have been described before in toxicological studies at extremely high dosages of ACEi or Angiotensin II receptor antagonist[20,21], but we used a dosage well-established to render optimal anti-proteinuric effects and corresponding optimal protection against glomerulosclerosis[6,11]. The current data show that this therapeutically relevant dose is associated with tubulointerstitial adverse effects despite better reduction of proteinuria and blood pressure.

Volume depletion is a well-known risk factor for acute renal failure during RAAS blockade in man. This is attributed to loss of efferent vascular tone, and is reversible upon volume repletion, provided no tubular necrosis has ensued. Long term exposure to the combination of low perfusion pressure and ACEi on the other hand, as reported in rats with unilateral renal artery stenosis, can result in renal abnormalities that are not reversible upon restoration of perfusion pressure, namely pronounced tubulointerstitial fibrosis, which can be considered in line with our current data[26,27].

Tubulointerstitial hypoxia is a plausible candidate mechanism for the observed changes. The tubulointerstitial lesions were predominantly localized in the inner cortex and outer medulla, i.e. the intra-renal region most vulnerable to hypoxia. Hypoxia might be elicited by the combination of a low perfusion pressure and increased tubular oxygen demand due to increased sodium reabsorption by volume depletion. Moreover, hypoxia is a potent stimulus for cellular proliferation and OPN expression[22-25]. Since OPN is not a specific for hypoxia, we also measured HO-1, which was upregulated in ACEi/LS rats only and compatible with hypoxia as a trigger for the interstitial lesions in ACEi/LS rats.

Another explanation for the adverse effects on the tubulointerstitium might be a disruption of the RAAS by ACEi. Pharmacological or genetic disruption of the RAAS during development results in renal interstitial and vascular abnormalities[28-35] mimicking those in our study. Intact AT1 receptor signaling is a prequisite for normal renal development and might be involved in maintenance of normal structures in the adult kidney under specific conditions, by the role of angiotensin II as a growth factor.

A common factor in both the above mentioned knock-out animals and during ACEi, in combination with low salt intake, is the high plasma renin activity. Renin knock-out mice, however do not develop these typical preglomerular vascular lesions strongly suggesting

involvement of renin in the development of the vascular changes[36]. Recent findings suggest that renin can induce renal alterations through activation of the pro-renin receptor[37,38]. If this mechanism would be involved (additional) RAAS blockade by renin inhibition might prevent the vascular changes induced by ACEi.

We observed marked afferent arteriolar wall thickening during ACEi which were most pronounced in LS/ACEi rats. Interruption of the negative feedback loop between angiotensin II and renin secretion in the juxtaglomerular cells is probably involved in these vascular changes. ACEi induces renin release accompanied by an increase in plasma renin activity[39], that is potentiated by low sodium. Moreover, rats and mice given ACEi develop thickening of afferent arterioles, i.e. hyperplasia of juxtaglomerular cells[40-43], which has been ascribed to a phenotypic change of contractile smooth muscle cells to renin containing epithelioid cells[44,45]. It would be of interest to investigate the functional consequences of these phenotypic changes for the regulation of downstream blood supply. For instance, whether loss of contractile properties might interfere with the homeostatic regulation of interstitial blood supply during conditions of low perfusion pressure. Our study, however, does not allow conclusions on the functional consequences of the observed vascular changes.

What could be the implications of our findings for renoprotective intervention? Sodium restriction enhances the effects of ACEi on the intermediate parameters proteinuria and blood pressure. Our data demonstrate that these findings cannot simply be extrapolated to enhanced protection against renal structural damage, as dissociation between the effects on intermediate parameters and renal structural damage can occur. Considering the consistent prognostic impact of interstitial damage for long term renal outcome, and the trend towards lower target blood pressure in proteinuric populations, these treatment-associated adverse renal effects may well have the potential to adversely affect long term outcome. Further studies should elucidate the mechanisms underlying these adverse renal effects of the combination of ACEi with low sodium, and their impact on long term renoprotection.

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